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Temperature and Oxygen-Nitrogen Gas Ratios Affect Fish Survival in Air-Supersaturated Water

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Effects of different oxygen-nitrogen gas ratios on fish mortality at the same total dissolved gas pressure in supersaturated water were demonstrated with juvenile steelhead trout. Mortality was rapid (time to 50% death in 1-6 h) at 140, 135 and 130% saturation, with fish dying more rapidly as the ratio of oxygen-nitrogen decreased (decrease in O_2, increase in N_2. Mortality patterns were similar at 125%; time to 50% death occurred in 5-20 h, with more rapid deaths occurring as oxygen (O_2/N_2 ratio) was decreased.

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TEMPERATURE AND OXYGEN–NITROGEN GAS RATIOS AFFECT FISH SURVIVAL IN AIR-SUPERSATURATED WATER

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Abstract—Juvenile steelhead trout and juvenile chinook, coho and sockeye salmon were tested at different temperatures (8°, 9°, 10°, 12°, 15°, 18° and 20°C) at the same concentration of air-supersaturated water. Supersaturated water concentrations in different tests were 115, 116, 117, 118 and 120% saturation. Increased temperatures caused a significant ($P < 0.005$) increase in steelhead mortality, a significant increase ($P < 0.025$) in chinook deaths, but no significant effect on coho or sockeye mortality. Regression model data for steelhead indicate that a 10°C increase in temperature will decrease the time to 50% death by a factor of 2.7, e.g. from 190 h at 8°C to 70 h at 18°C, when tested at the same total dissolved gas pressure.

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INTRODUCTION

The varying solubility of gases in water at different temperatures is well known, and temperature tolerances and oxygen requirements of a variety of fishes and aquatic organisms are known for a wide range of conditions and with some toxic substances (Coutant & Talmadge, 1977). However, there is little information on the effects that temperature or varying oxygen–nitrogen gas ratios may have on the lethality of air-supersaturated water to fish and other aquatic life. An increase in the temperature of saturated water will supersaturate the water with gases (Shelford & Allee, 1913; DeMont & Miller, 1971), but little is known of the effects when fish are subjected to the same concentration of air-supersaturated water at different temperatures. Three thermal studies have considered some aspects of the interaction of temperature and supersaturation (Coutant & Genoway, 1968; Ebel *et al.*, 1971; Bouck *et al.*, 1976). Fickeisen *et al.* (1976) determined effects of different temperatures on black bullhead (*Ictalurus melas*) survival at the same gas levels. They found little or no effect of supersaturation on the tolerance of the fish at temperatures of 8°, 12°, 16° and 20°C. Marcello *et al.* (1975) showed that there were no significant effects of temperature on the survival of menhaden (*Brevoortia tyrannus*) at different supersaturation concentrations.

The initial work on effects of varying oxygen and nitrogen gas ratios in supersaturated water by Rucker (1976), using juvenile coho salmon, and the work by Nebeker *et al.* (1976*a*) with juvenile sockeye salmon, showed that varying gas composition of supersaturated water can affect fish survival. Nitrogen gas, which forms a significant portion of the total dissolved gas pressure, is a major factor causing mortality, though oxygen does cause severe external signs of gas bubble disease. Rucker used 119% total gas saturation and varied the O_2/N_2 ratios. Nebeker *et al.* used 120, 125 and 130% total saturation with several O_2/N_2 ratios. The two papers summarize other relevant literature that need not be cited again here.

The purpose of the present study was to determine if different temperatures might have an effect on the survival of juvenile salmon and steelhead at a constant supersaturation concentration and to present data for juvenile steelhead trout (*Salmo gairdneri*) tested at several total dissolved gas pressure levels and O_2/N_2 ratios.

METHODS AND MATERIALS

Fish

Steelhead trout smolts (*Salmo gairdneri*), and juvenile chinook (*Oncorhynchus tshawytscha*), coho (*O. kisutch*), and sockeye salmon (*O. nerka*) were reared from eggs at the Western Fish Toxicology Station. Fish were fed daily with Oregon Moist Fish Diet and were acclimated to test temperature for at least one week before testing, with rates of temperature change not exceeding 1.5°C/day. Steelhead used for temperature tests were tested at ~15 months of age during the time they were smolts and would nor-

Table 1. Times to 50 and 20% death for juvenile steelhead trout and juvenile sockeye, chinook and coho salmon at different gas saturation levels and water temperatures. Each value represents time to 50% death, using 20 fish for each test tank

Test number	Mean measured % total gas saturation*	Nominal water temp. (°C)	Time to 50% death (h) steelhead	sockeye	chinook	coho	Time to 20% death (h) steelhead	sockeye	chinook	coho
1	119.6	8	102	45	82	235	70	29	47	152
	119.8	12	84	51	84	‖	61	30	55	195
	119.7	16	35	63	198	‖	27	37	54	160
	119.3	20	40	57	53	270	28	34	40	51
2	114.3	10	510, ‖†	‖, ‖	—	—	320, 380	‖	—	—
	114.5	12	505, 408	‖, ‖	—	—	285, 215	‖	—	—
	114.8	15	268, 305	‖, ‖	—	—	150, 156	‖	—	—
	114.7	18	202, 258	‖, ‖	—	—	107, 135	‖, 480	—	—
3	116.1	9	462, 223	515, 418	—	—	175, 108	165, 177	—	—
	116.2	12	242, 252	395, 525	—	—	141, 158	214, 205	—	—
	116.2	15	193, 118	490, 470	—	—	92, 57	154, 173	—	—
	116.4	18	72, 52	313, 453	—	—	39, 37	162, 212	—	—
4‡	116.5	9	160, 193	287, 456	—	—	101, 127	116, 158	—	—
	116.8	12	211, 183	397, 603	—	—	88, 122	122, 195	—	—
	117.0	15	178, 143	‖, ‖	—	—	93, 87	272, 320	—	—
	116.8	18	102, 113	‖, ‖	—	—	55, 54	‖, ‖	—	—
5	120.4	9	45, ‖	‖, ‖	—	—	29, 35	‖, 30	—	—
	120.8	12	44, 40	‖, ‖	—	—	28, 27	43, 39	—	—
	120.6	15	43, 40	49, 36	—	—	30, 28	34, 21	—	—
	121.8§	18	—	—	—	—	—	—	—	—
6	120.2	10	56	49	90	43	31	20	46	30
	120.9	12	33	46	55	41	20	23	31	26
	120.3	15	42	‖	50	55	27	41	29	31
	119.6	18	32	37	31	46	24	22	22	30
7	117.2	9	121	226	440	230	56	128	200	100
	117.8	12	123	250	311	276	73	131	220	156
	117.5	15	96	216	235	319	76	104	145	172
	117.6	18	62	332	205	136	45	105	94	58

* Control tanks remained near 18°C and 100% saturation at all times.

† Two tests were completed at each temperature.

‡ The formula $\frac{BP + \Delta P}{BP} \times 100 = \%$ sat. was used for this test only. Other tests used $\frac{BP + \Delta P - VP}{BP} \times 100 = \%$ sat.

§ Saturation level significantly higher than other tanks, data not used.

‖ Insufficient deaths to achieve 50 or 20% mortality.

mally be migrating down to the ocean. Average fork length was 15 cm; mean blotted wet wt was 32 g. Mean steelhead size when tested in mixed gases ranged from 5.8 g (7.9 cm) to 40.5 g (16.2 cm), depending upon age and time of year tested. Chinook ranged from 23.8 g (12.6 cm) to 95.8 g (20.6 cm), sockeye from 6.6 g (8.4 cm) to 111.5 g (20.9 cm), and coho from 20.5 g (11.9 cm) to 51.6 g (16.7 cm), again depending on age and time of year tested.

Water and test facility

Unchlorinated, aerated well water was used for all testing and had the following average characteristics: hardness (as $CaCO_3$)—31 mg l^{-1}; alkalinity (as $CaCO_3$)—23 mg l^{-1}; pH—7.1. Water chemical analyses were conducted according to the American Public Health Assoc. *et al.* (1971). Water temperature, velocity and flow rate were controlled, and natural photoperiod was utilized during testing.

Test facilities consisted of five 6000-l. fiberglass tanks, each with a water depth of 60 cm and containing a four-chambered nylon net cage for testing 4 groups of fish at the same concentration or temperature. Each tank had a separate supersaturation generator (Nebeker *et al.*, 1976*b*) where water was supersaturated by injecting compressed air, or air and O_2 or N_2 gas, into water under pressure and then releasing the water into the test tanks. The per cent saturation was controlled by the amount of air (and gases) metered into the test water. Because supersaturation of gas in water increases with elevated temperature, a different amount of gas had to be added at each temperature to maintain the same total per cent saturation. A Van Slyke gas analyser, the Winkler method for dissolved oxygen, and a Weiss saturometer were used to determine saturation concentrations. The formula $BP + \Delta P - VP/BP \times 100 = \%$ saturation, where BP = atmospheric pressure, ΔP = saturometer reading, and VP = water vapor pressure, was used to calculate total dissolved gas pressure with the Weiss saturometer. Per cent oxygen and nitrogen (+argon) saturation values were determined using the methods and calculations outlined by Nebeker *et al.* (1976*a*).

Temperature testing

Fish were tested at 8°, 9°, 10°, 12°, 15°, 18° and 20°C at total gas supersaturation concentrations of 115, 116, 117, 118 and 120% (Table 1). In each tank of supersaturated water one or two tests were conducted at each temperature with one test cage per species, 20 fish per cage. After acclimation to test facilities and temperatures, the water was supersaturated, requiring about 8 h to reach test levels. The fish were in the test water while supersaturated conditions

These data indicate the likelihood of increased mortality of steelhead trout and chinook salmon juveniles in supersaturated water at higher temperatures. Apparently coho and sockeye are not significantly affected, as long as the temperatures are below those which do not directly cause temperature-related mortality. The supersaturation concentrations used during this study are near the 96-h LC50 values (50% mortality after 96 h) determined from previous studies (Bouck *et al.*, 1976) and have been shown to occur in the Columbia and Snake Rivers (Ebel & Raymond, 1976). Regression data for steelhead trout in this study indicated that a 10°C decrease in temperature will increase the LT50 by a factor of 2.72; if the LT50 for 118% saturation at 18°C was 70 h it would be near 190 h at 8°C (Fig. 1). There was no significant difference in LT50 at different temperatures for sockeye juveniles.

As mentioned in the Methods section, the formula used to calculate supersaturated water concentrations subtracted water vapor from the saturometer reading. In test No. 4 water vapor was not subtracted during calculations to see if this would result in survival data different from the other 6 tests. It can be seen (Table 1) that similar mortality would occur with either method of calculating supersaturated water concentrations.

It is apparent from this study that the temperature of supersaturated water may have an effect on fish survival, depending on species and total dissolved gas pressure. Therefore, water temperature should be considered when determining if levels of supersaturation are safe for a fish species in a given water system. This apparent biological difference due to temperature increase is different from the physico-chemical increase in supersaturation when water is heated and becomes supersaturated. In general, air supersaturation in water will increase 2.5% for every 1°C rise in water temperature, i.e. water will be supersaturated to 125% if temperature is raised from 5° to 15°C.

When the temperature of a water system increases, either naturally from solar insolation or from thermal springs, artificially due to man's activities, or due to a combination of both, the resultant temperature may have a deleterious effect on fish populations. If the water is supersaturated by spillage over dams and subsequently heated, fish and invertebrate populations would be seriously threatened, if not completely eliminated from affected areas.

Mixed gas tests

As in the two earlier studies by Rucker (1976) with coho salmon and Nebeker *et al.* (1976) with sockeye salmon, the present study with steelhead showed that there was a significant difference ($P < 0.05$) in time to death at different O_2/N_2 ratios when fish were tested at the same total dissolved gas pressure. Fish died rapidly at 140 and 135% saturation, with fish dying more quickly at the lower oxygen–nitrogen ratios where nitrogen made up a much greater portion of the total gas. Mortality also occurred more rapidly at the lower O_2/N_2 ratios at 130%. Mortality patterns at 125% were similar to the other three total gas concentrations but the LT50 increased as the oxygen concentrations increased (Fig. 2). The lower oxygen concentrations in the four tests: 4.8 mg l^{-1} at 125%, 4.9 mg l^{-1} at 130%, 4.4 mg l^{-1} at 135% and 6.1 mg l^{-1} at 140% may have contributed to the mortality caused by supersaturation, but this was not determined due to the rapid mortality at the high nitrogen levels needed to maintain the desired total gas pressures.

In plotting the effects of different O_2/N_2 ratios on the survival of steelhead (time to 50% death) at the same total dissolved gas pressure, it was found that the same results could be obtained using dissolved oxygen rather than O_2/N_2 ratios, making the graphs simpler to prepare and understand. The curves in Fig. 2 show, based on the data available, what the time to death might be at different O_2 levels and total dissolved gas pressures. We were unable to construct a reasonably simple mathematical model which fits the patterns found in Fig. 2.

The curve (X) showing time to 50% mortality for juvenile coho salmon from Rucker (1976) is superimposed on Fig. 2 to see how it compares with the data in this paper. Although the mortality pattern was similar, the time to death cannot be compared because more tolerant coho salmon were used in the Rucker study, and steelhead trout were used in the present study.

At 125% total gas saturation steelhead died most rapidly at the lowest O_2 concentration of 4.8 mg l^{-1} (O_2/N_2 ratio of 44.2/146.6), indicating that the low oxygen–high nitrogen combination was rapidly lethal, with 50% mortality occurring at 4.7 h exposure. At the highest O_2 concentration of 14.3 mg l^{-1} (O_2/N_2 ratio of 129.4/125.6), the LT50 was 16.5 h, 4 times as long as the lower O_2/N_2 ratio. The low O_2 concentration of 4.9 mg l^{-1} (O_2/N_2 ratio of 45.9/151.8) at 130% had 50% mortality at 3.7 h, while the high O_2 concentration of 18 mg l^{-1} (O_2/N_2 ratio of

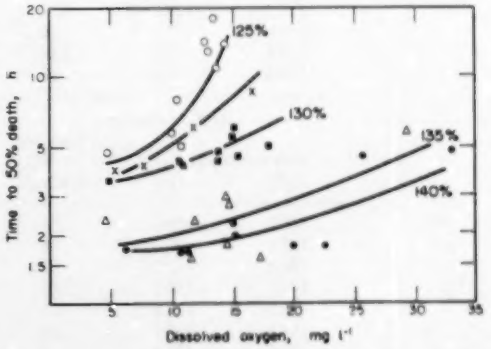

Fig. 2. Effects of varying O_2/N_2 ratios at 125, 130, 135 and 140% total dissolved gas pressure on steelhead trout survival. Data of Rucker (X) showing times to 50% mortality are superimposed on Fig. 1.

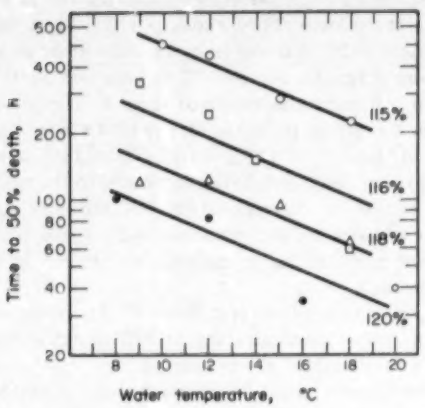

Fig. 1. Effect of different exposure temperatures on the survival of juvenile steelhead trout at four different concentrations of supersaturated water.

were being produced. Fish were observed for mortality frequently during the day and evening, especially during tests involving higher concentrations. Time to 50% death (LT50) was determined by plotting cumulative mortality against time on log-probit paper. A straight line was fitted through the points and where it crossed the 50% mortality mark was considered the time to 50% mortality (LT50) for that group of 20 fish. The slopes of the lines (Fig. 1) were not significantly different so they were set equal to each other (regression of log (LT50)).

Mixed gas testing

Fish were tested at total gas supersaturation concentrations of 125, 130, 135 and 140% total dissolved gas with 37 different oxygen–nitrogen (O_2/N_2) ratios, ranging from 40% (4.4 mg/l) to 307% (33.1 mg/l) O_2 saturation; and 96–162% nitrogen saturation (Table 2). In each tank of supersaturated water, two or more tests were completed at each O_2/N_2 gas ratio and supersaturation concentration, with 10 or 20 fish used during each test. Fish were under continuous observation until greater than 50% had died. Fish were transferred directly to the supersaturated water from holding tanks at the same temperature (12°C), rather than being placed in the tanks prior to supersaturating the water. Time to 50% death (LT50) was determined in the same manner as in temperature testing.

RESULTS AND DISCUSSION

Temperature tests

The effect of different temperatures and constant supersaturation concentration on survival of the juvenile steelhead and salmon varied with species (Table 1). Increased temperatures caused a very significant ($P < 0.005$) increase in steelhead mortality, a significant ($P < 0.025$) increase in chinook deaths, but no significant effect on coho or sockeye mortality. Using regression models, highly significant ($P < 0.001$) and significant ($P < 0.005$) temperature effects were shown on steelhead and chinook, respectively. For sockeye and coho, such regressions did not show a significant effect of temperature for either the LT50 or the LT20.

Table 2. Summary of mean time to 50% death for juvenile steelhead trout at 125, 130, 135 and 140% total dissolved gas pressure and varying O_2/N_2 ratios

125% total gas			130% total gas		
Gas concentration O_2%	Gas concentration N_2%	Mean time to 50% death (h)	Gas concentration O_2%	Gas concentration N_2%	Mean time to 50% death (h)
*44.2	146.6	4.7	*45.9	151.8	3.7
93.0	133.4	5.9	99.2	138.1	4.2
95.7	133.5	8.2	100.4	138.3	4.5
99.5	132.2	5.0	128.4	131.1	5.0
117.4	128.1	16.5	129.2	130.8	4.2
120.2	125.9	16.7	139.0	128.0	5.5
123.8	125.3	12.0	140.0	128.2	6.0
123.8	125.8	20.0	140.0	127.8	5.0
129.4	125.6	16.5	166.4	121.1	5.5
135% total gas			**140% total gas**		
Gas concentration O_2%	Gas concentration N_2%	Mean time to 50% death (h)	Gas concentration O_2%	Gas concentration N_2%	Mean time to 50% death (h)
*40.5	159.6	2.5	56.6	162.4	1.7
107.8	142.3	1.6	99.0	151.9	1.9
110.1	143.0	2.3	104.1	150.3	1.7
131.7	137.3	3.0	138.2	141.4	2.2
135.3	136.1	2.7	140.9	140.7	2.0
135.6	135.3	1.8	187.1	129.1	1.7
161.2	128.9	1.5	210.0	122.1	1.9
272.5	99.4	5.7	237.9	118.0	4.5
			307.9	96.1	5.0

* Possible additional stress due to low oxygen.

166.5/121.1) had an LT50 of 5.5 h. The difference is not as great as that of 125% because the total gas concentration of 130% kills fish more rapidly. Table 2 gives a summary of the times to 50% death (LT50) for steelhead trout for each total gas concentration and each O_2/N_2 ratio.

A significant change in the ratio of oxygen to nitrogen in air-supersaturated water will result in a change in the lethality of that water to fish. If photosynthetic activity occurs, dissolved oxygen in the water will increase, the total dissolved gas pressure will increase, and the oxygen/nitrogen ratio will change, unless the water is vigorously agitated. The actual nitrogen concentration may decrease due to the physical stripping of nitrogen from the water column as oxygen bubbles rise to the water surface. In most instances water temperature increases during active photosynthetic activity because of increased insolation. This increased water temperature results in an increase in the total dissolved gas pressure, or supersaturation. If the temperature of the water increases without increased photosynthesis (from algae or higher aquatic plants) the oxygen–nitrogen ratio (O_2/N_2) may not change significantly though the total per cent supersaturation will increase. If the temperature stays the same during increased photosynthetic activity (e.g. an algal bloom), the O_2/N_2 ratio will increase due to greater amounts of oxygen being produced. If the temperature increases during an algal bloom, supersaturation levels and the O_2/N_2 ratio will increase, depending upon the rate of temperature and O_2 changes.

The other main cause of change in the O_2/N_2 ratio is biological respiration, or use of oxygen by algae, higher plants, bacteria, fishes, etc. This decreases oxygen in the water, lowering the O_2/N_2 ratio and the total dissolved gas pressure, or supersaturation in the water. Man may radically alter aquatic systems by introducing nutrients which stimulate algal growth or increase bacterial respiration, causing even greater fluctuation in the O_2/N_2 ratio.

The results of this study are consistent with those of Rucker (1976) and Nebeker *et al.* (1976). Increased total air supersaturation causes greater mortality and a reduction in the LT50. An increase in the amount of oxygen (decrease in nitrogen), keeping the total gas saturation level the same, will reduce the mortality and increase the LT50. Conversely, an increase in the nitrogen concentration (decrease in oxygen) keeping the gas saturation concentration the same, will increase the mortality and decrease the LT50. The work of Knittel *et al.* (1978) showed clearly that if fish are removed from supersaturated water prior to death they can recover. Fish and other aquatic life may be able to tolerate brief higher levels of supersaturation because increased water temperature and photosynthetic activity during the day are offset by cooling and high respiration rates at night. Further studies with intermittent exposures simulating the daily changes in total per cent saturation and O_2/N_2 ratios in supersaturated water would be useful in predicting extent of fish mortality in those situations.

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